

Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Canceled)
2. (Previously Presented) The method of claim 45 in which the amplification of step (i) is further carried out in the presence of a reverse transcriptase such that the polymerase chain reaction is reverse-transcription polymerase chain reaction and wherein the one or more polynucleotide sequences is obtained from mRNA derived from the sample.
3. (Previously Presented) The method of claim 45 in which the one or more polynucleotide sequences comprise a cDNA library.
4. (Canceled)
5. (Previously Presented) The method of claim 45 in which the polymerase chain reaction of step (i) is carried out for a number of cycles such that the amplification remains in the linear range.
6. (Previously Presented) The method of claim 45 in which the amplification in step (i) is achieved with a thermostable DNA polymerase.

7. (Canceled)

8. (Previously Presented) The method of claim 45 in which the label is a fluorophore.

9. (Previously Presented) The method of claim 45 in which said plurality of oligonucleotide probes is selected from the group consisting of 5'-exonuclease probes, stem-loop beacon probes and stemless beacon probes.

10. (Withdrawn). The method of claim 1 in which said at least one oligonucleotide probe comprises a plurality of oligonucleotide probes, each of which is complementary to a region of a different amplified target gene sequence of interest.

11. (Withdrawn) The method of claim 10 in which the product of step (i) is divided into a plurality of aliquots and said quantifying in step (ii) is performed on said aliquots.

12. (Withdrawn) The method of claim 11 wherein the number of aliquots is equal to the number of primer pairs used in the multiplex amplification.

13. (Withdrawn) The method of claim 12 in which step (ii) comprises amplifying the

product in each aliquot by polymerase chain reaction in the presence of an amplification primer set suitable for amplifying one of the target sequences of the plurality.

14. (Withdrawn) The method of claim 13 in which the amplifying in step (ii) is further carried out in the presence of an oligonucleotide probe complementary to a region of a different amplified target gene sequence of interest, wherein each probe in step (ii) comprises one of the oligonucleotide probes in step (i).

15. (Withdrawn) The method of claim 12 in which the sequences of the amplification primer sets of step (i) are the same as the sequences of the amplification primer sets of step (ii).

16. (Withdrawn) The method of claim 11 in which the amplifying in step (ii) is further carried out in the presence of a molecule that produces a detectable signal when bound to a double-stranded polynucleotide suitable for monitoring the amplification reaction as a function of time.

17. (Withdrawn) The method of claim 16 in which the molecule is selected from the group consisting of an intercalating dye and a minor groove binding dye.

18. (Withdrawn) The method of claim 17 in which the molecule is selected from the group consisting of SYBR® green I and ethidium bromide.

19 - 21. (Canceled)

22. (Previously Presented) The method of claim 45 in which an observed efficiency of amplification is greater than 70%

23. (Previously Presented) The method of claim 45 in which an observed efficiency of amplification is greater than 90%.

24 - 42. (Canceled)

43. (Previously Presented) The method of claim 45 in which the amplification is carried out in the presence of uracil N-glycosylase.

44. (Previously Presented) The method of claim 45 in which the amplifying the at least one polynucleotide sequences comprises as many as fourteen PCR cycles.

45. (Previously Presented) A method for analyzing a sample or plurality of samples for the presence of one or more polynucleotide sequences of interest, comprising:

(i) amplifying at least one of said polynucleotides derived from said sample or said plurality of samples in the presence of:

(a) a plurality of different amplification primer pairs suitable for amplifying said polynucleotide sequences of interest; and

(b) a plurality of oligonucleotide probes, wherein each of said plurality of oligonucleotide probes is complementary to a region of a different polynucleotide sequence of interest amplified by said plurality of primer pairs and comprises a label suitable for monitoring amplification as a function of time; and

(ii) amplifying the products of said step (i) by dividing said products of step (i) into a plurality of aliquots and performing real-time PCR of at least one of said aliquots in the presence of:

(a) at least one of said primer pairs used in step (i); and

(b) at least one of said oligonucleotide probes used in step (i) wherein said oligonucleotide probe in step (ii) is complementary to a region of a polynucleotide sequence of interest amplified by said primer pair in step (ii).